REMARKS

Claims 16, 17, 19, 20, 22, and 33 – 38 are pending in the application. Claim 33 has been cancelled. Claims 16, 19, 22, 34 - 38 have been amended. No new claims have been added. Support for the amendments to the claims and for the new claims can be found in the specification and claims as originally filed. No new matter has been added.

Objection to the Specification

The Examiner has objected to the Specification because the filing date and Accession number for the cell referred to as DPL are missing on page 8, lines 26 and 27. Applicants have amended the specification and respectfully request withdrawal of the objection.

Rejection of Claims 16, 17, 19, 20, 22 and 33 – 38 Under 35 USC 112, First Paragraph

The Examiner has rejected claims 16, 17, 19, 20, 22 and 33 - 38 under 35 USC 112, first paragraph. The Examiner argues that the specification contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the claimed invention. (Office Action, p.2 – 3). Applicants respectfully traverse the rejection.

The instant claims recite a method of killing a cell that is sensitive to DT-A or PEA, comprising infecting the cell with an adenovirus produced by a packaging cell line, wherein the cell line is capable of producing adenovirus that expresses the A subunit of diphtheria toxin (DT-A) or Pseudomonas Exotoxin A (PEA), wherein the cell line does not produce replication-competent adenovirus when used in conjunction with non-overlapping E1-deleted adenovirus, wherein the cell line is resistant to DTA and PEA and wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705 (Claim 16).

The Examiner points out that "the specification teaches making a cell line comprising a human EF-2 gene encoding an EF-2 protein that is mutated at codon 705." (Office Action, p.3). However, the Examiner argues that in view of the lack of guidance in the specification and prior art, the skilled artisan would have to further experiment to determine whether or not other EF-2 genes encoding an EF-2 protein mutated at codon 705 possess the desired biological activity" (Office Action, p.3).

The instant claims recite a cell line that has a mutated **human** EF-2 gene that encodes an EF-2 protein that is mutated at codon 705. In view of the amendments to the claims, Applicants submit that Applicants were in possession of the claimed invention at the time of filing the application.

Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the foregoing rejection.

Rejection of Claims 16, 17, 19, 20, 22 and 33 – 38 Under 35 USC 112, First Paragraph

The Examiner has rejected claims 16, 17, 19, 20, 22 and 33 - 38 under 35 USC 112, first paragraph. Specifically, the Examiner believes that the specification "while being enabling for a method of killing a cell in vivo using direct delivery of the adenovirus to the cell and a method of killing a cell in vitro, does not reasonably provide enablement for a method of killing a cell in vivo using a genus of administration routes." (Office Action, p.5). Applicants respectfully traverse the rejection.

The instant claims have been set forth herein.

The Examiner alleges that "(t)he claimed invention embraces using a genus of administration routes to a cell in vivo." (Office Action, p.5). The Examiner argues that "it would take one skilled in the art an undue amount of experimentation to determine what route of administration...other than direct administration and/or systemic administration would result in a therapeutic response using a vector embraced in the claims." (Office Action, p.9). The Examiner argues further that "the applicants teach IJ or IP were suitable administration routes for delivering an adenovirus comprising the claimed nucleic acid into the liver of mice infected with HCV (but) the skilled artisan cannot reasonably extrapolate from the results using an adenovirus to a genus of vectors because each vector has a different mechanism and tropism." (Office Action, p.9). The Examiner alleges that "the instant specification and claims coupled with the art of record...only provide enablement for an in vitro method of suppressing growth of a cancer cell and an in vivo method of suppressing growth of a cancer cell in a subject comprising direct administration to the cancer cell and not for the full scope of the claimed invention." (Office Action, p.9).

For enablement purposes, a specification need not teach what is well known in the art. In re Wands, 858 F.2d 731 (Fed. Cir. 1988). Moreover, the need for some amount of experimentation is not fatal as long as the amount is not undue. Id. For the instant claims, no experimentation is required, because the specification provides sufficient guidance to

allow one having ordinary skill in the art to make and use an adenovirus produced by a packaging cell line, wherein the cell line is capable of producing adenovirus that expresses the A subunit of diphtheria toxin (DT-A) or Pseudomonas Exotoxin A (PEA), wherein the cell line does not produce replication-competent adenovirus when used in conjunction with non-overlapping E1-deleted adenovirus, and wherein the cell line is resistant to DTA and PEA and wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705 according to the claimed methods. More specifically, the disclosure details how to make DT resistant cells (e.g., p.11, line 20; p.12 - 13, example 1). Moreover, methods for the production of adenovirus using packaging cells as used in the methods of the invention are known in the art, as pointed out on page 9, line 14 of the disclosure.

Applicant submits that there is no requirement that Applicant provide data for every therapeutic method (see Amgen v. Chugai and Genetics Institute, 927 F.2d 1200 (Fed. Cir. 1991)). It is well established that examples are not required for an enabling disclosure. In re Robins, 166 U.S.P.Q. 552 (C.C.P.A. 1970); In re Borkowski, 164 U.S.P.Q. 642 (C.C.P.A. 1970). The first paragraph of 35 U.S.C. § 112 requires nothing more than objective enablement, which Applicant has provided. Thus, the examples included in the present application should be considered as supportive of enablement.

It is also respectfully submitted that a person having ordinary skill in the art, can take the adenovirus produced by the methods of the invention as set forth in the disclosure for administration to a subject by any method well known in the art, provided that they are amenable to the administration of adenovirus.

For example, the work of Maria Castro et al. has recently demonstrated intracranial adenoviral delivery of Pseudomonas Exotoxin (PE) in an in vivo model. It is noted that Psuedomonas Exotoxin has the same catalytic activity as Diphtheria toxin, the difference being that one toxin has the catalytic subunit at the amino terminus and the other at the carboxy terminus. However, both Pseudomonas Exotoxin and Diptheria toxin cause ADP ribosylation at the diphtherimde residue in EF-2. Castro et al. demonstrate that adenoviral-mediated delivery of Pseudomonas Exotoxin (PE) Fused to IL-13 induces regression of intracranial human glioblastoma xenografts in mice (a copy of the abstract presented at the American Society for Gene Therapy 10th Annual Meeting is enclosed herewith (Seattle, WA. Simultaneous Oral Abstract Sessions: Cancer Gene Therapy (10:15 AM-12:15 PM), Saturday June 2, 2007)).

Therefore, contrary to the Examiner's assertion, the methods of the present invention are enabled for methods of delivering the adenovirus as described herein.

In further support of the contention that the claims are not enabled, the Examiner cites the Verma et al., McNeish et al. and Vile et al. references, allegedly disclosing problems with gene therapy. These references describe general concepts regarding gene therapy. In this regard, to date there are dozens of clinical trials in the U.S., and many more around the world, that involve the use of gene therapy. Thus, while failures may occur, it is important to consider the successes that have occurred in the field of gene therapy.

For example, Applicant wishes to draw the Examiner's attention to the results of gene therapy to treat severe combined immunodeficiency, as disclosed by Blaese et al. (Science 270:475-480 (1995). In this study, two children with a genetic defect in production of adenosine deaminase (ADA) were treated with a cloned ADA gene inserted into a retroviral vector. To this day both patients continue to display significant improvement in their immune system function. The results of this gene therapy treatment were markedly superior to those produced earlier by alternative treatment means.

In a cancer context, Roth et al. (Nature Medicine 2(9):985-991 (1996)) have shown that a recombinant retroviral vector targets tumor cells in vivo. Moreover, this vector, which encodes the tumor suppressor p53, provided a sufficient level of p53 expression such that apoptosis, or programmed cell death, was triggered in these cells. Accordingly, retrovirus gene therapy was accomplished in vivo. Khuri et al. (Nature Medicine 6(8):879-885 (2000)) reported a successful gene therapy regimen in human cancer patients using ONYX-015, an oncolytic, chimeric group C adenovirus having a large deletion in the E1B gene.

With respect to X-linked severe combined immunodeficiency (i.e., SCID-X1), Cavazzana-Calvo et al. (Science 288:669-672 (2000)), have demonstrated full correction of disease phenotype in patients treated by gene therapy protocols. Further, Kay et al. (Nature Genetics 24:257-261 (2000)) have demonstrated therapeutic efficacy in the treatment of Haemophilia B with AAV vectors carrying the gene that encodes factor IX.

The successes of gene therapy are not limited to only these examples. According to a 1995 review article (Crystal, Science 270:404, 405 (1995)),

> [p]robably the most remarkable conclusion drawn from the human trials is that human gene transfer is indeed feasible . . . [and] most studies have shown that genes can be transferred to humans whether the strategy is ex vivo or in vivo, and that all vector types function as intended.

Clearly, there is evidence to support successful human gene transfer in both ex vivo and in vivo studies.

Applicants respectfully request that the Examiner reconsider and withdraw the foregoing rejection.

Rejection of Claims 35 - 38 Under 35 USC 112, Second Paragraph

The Examiner has rejected claims 35 - 38 under 35 USC 112, second paragraph as being indefinite. Specifically, the Examiner argues that the limitation "the cells" lacks the proper antecedent basis.

Applicants have amended the claims as suggested by the Examiner to recite the limitation "the packaging cell lines."

Accordingly, Applicants respectfully request withdrawal of the rejection and allowance of the claims.

Rejection of Claims 16, 17, 19, 20, 22 and 35 - 38 Under 35 USC 103

The Examiner has rejected claims 16, 17, 19, 20, 22 and 35 - 38 under 35 USC 103(a) as being unpatentable over Rodriguez et al. (Proceedings of the American Association for Cancer Research 37, 346:2358, 1996) taken with Leiber et al (US 6,686,196) and Fallaux et al. (US 5,994,128) and further in view of Horlick et al. (US 6,647,002). Applicants respectfully traverse the rejection.

The claims as amended are directed to methods of killing cells that are sensitive to DT-A or PEA, by infecting the cells with an adenovirus produced by a packaging cell line, wherein the cell line is capable of producing adenovirus that expresses the A subunit of diphtheria toxin (DT-A) or Pseudomonas Exotoxin A (PEA), wherein the cell line does not produce replication-competent adenovirus when used in conjunction with non-overlapping E1-deleted adenovirus, wherein the cell line is resistant to DTA and PEA and wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705 (see claim 16).

The Examiner argues that the Rodriguez reference "teaches that a nucleic acid encoding DT or PEA can be used in cancer gene therapy using an adenovirus." (Office Action, p.12). The Examiner admits that "Rodriguez does not specifically teach the methods steps for practicing a method of cancer gene therapy." (Office Action, p.12).

The primary reference, Rodriguez et al., does not teach or suggest infecting the cell with an adenovirus produced by a packaging cell line, **wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705**.

The use of an adenovirus produced by a packaging cell line, wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705 would not have been contemplated by the ordinary skilled artisan at the time the application was filed. None of the secondary references, Leiber et al., Fallaux et al. and Horlick et al., make up for the deficiencies of the Rodriguez reference. Nowhere in the Leiber et al., Fallaux et al. and Horlick et al. references is there teaching or suggestion of an adenovirus produced by a packaging cell line, wherein the cell line has a mutated human EF-2 gene that encodes an EF-2 protein that is mutated at codon 705.

Accordingly, the cited references would not lead one of ordinary skill in the art to the claimed invention.

Applicants respectfully request that the Examiner reconsider and withdraw the foregoing rejection.

CONCLUSION

Applicants believe that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

Applicants submit herewith a petition for a three-month extension of time extending the deadline for a response to January 25, 2008.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Dated: January 25, 2008

Respectfully submitted,

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Adenoviral-Mediated Delivery of *Pseudomonas* Exotoxin (PE) Fused to IL-13 Induces Regression of Intracranial Human Glioblastoma Xenografts in Mice

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Human Glioblastomas (GBM) overexpress an IL-13 receptor (IL13á2R) that is absent in the normal brain. Thus, attempts have been made to target toxic molecules to glioma cells by fusing them with IL-13. However, protein preparations have a short half life, requiring frequent administrations. Thus, we constructed an adenoviral vector (Ad) to transfer a chimeric toxin composed by IL-13 and *Pseudomonas* exotoxin A (IL13-PE) to preclinical GBMs. We constructed Ad-IL4-*TRE*-IL13-PE that expresses IL-13-PE, and, as a safety feature, it also expresses a mutated form of IL4 that blocks the physiological receptor, i.e., IL13/IL4R. Transgene expression is driven by the bidirectional TRE promoter, which is activated by the transactivator (TetON, expressed within Ad-TetON), in the presence of the inducer doxycyline (Dox). IL4 ands IL13-PE expression was detected using human U251 glioma cells infected with Ad-IL4-*TRE*-IL13-PE+Ad-TetON, which reduced cell viability 70% in the "ON" state (+Dox). Human glioma cell viability remained unaffected in the "OFF" state, indicating that the expression of the chimeric toxin can be tightly regulated. Transgene expression from Ad-IL4-*TRE*-IL13-PE was also detected in COS-7 cells. However, COS-7 cells, which do not express the IL13alpha2R, did not undergo cell death in the presence of the therapeutic virus, suggesting that IL13-PE cytotoxicity is specific to glioma cells. We also administered Ad-IL4-*TRE*-IL13-PE+Ad-TetON in the striatum of nude mice, which were fed chow containing Dox. IL-4 and IL13-PE toxin were readily detected in the mouse brain, with no signs of toxicity. We then administered Ad-IL4-*TRE*-IL13-PE+Ad-TetON intratumorally into intracranial human U251 GBM xenografts in nude mice. While saline-treated mice (median survival: 45 days) all the animals treated with Ad-IL4-*TRE*-IL13-PE survived for over 100 days post-tumor implantation. Our results suggest that Ad-mediated intratumoral expression of IL13-PE toxin will lead to effective cytotoxicity of IL-13á2R expressing-GBM cells without side effects to the surrounding normal brain and warrant further development of this approach for the implementation of a clinical trial for GBM. *Supported by: National Institutes of Health/National Institute of Neurological Disorders & Stroke (NIH/NINDS) Grants 1R01 NS44556.01, Minority Supplement NS445561; 1 R21 NS054143-01 and 1 RO3 TW006273-01 to M.G.C.; NIH/NINDS Grants 1 RO1 NS 42893.01; U54 NS045309-01, and 1R21 NS047298-01 to PRL; the Bram and Elaine Goldsmith and the Medallions Group Endowed Chairs in Gene Therapeutics to PRL and MGC, respectively, and The Linda Tallen & David Paul Kane Foundation and the Board of Governors at CSMC.*

Keywords: Adenovirus; Apoptosis; Cancer Gene Therapy

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